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(54) Title: THE H-Y ANTIGEN

(57) Abstract

H-Y is a transplantation antigen that can lead to rejection of HLA-matched male organ and bone marrow grafts by female recipients, and may play a role in pregnancy and spermatogenesis. However, the origin and function of H-Y antigens has eluded researchers for 40 years. We show that one human H-Y peptide antigen presented by HLA-B7 is an 11 residue peptide derived from SMCY, an evolutionarily conserved Y chromosomal protein. A homologous gene on the X chromosome, SMCX, differs by two residues in the same region. We also show a peptide antigen recognized by two HLA-A2.1 restricted T cell clones, which is also encoded by SMCY. The identification of H-Y offers prospects for improvements in transplantation outcome, prenatal diagnosis and fertilization strategies.

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Title: The H-Y antigen.

The invention relates to the field of immunology, in particular to the field of cellular immunology.

It is also concerned with the area of organ transplantation, grafting of tissues or cells, especially bone marrow and possible immunological reactions caused by transplantation and/or grafting and bloodtransfusion.

Since the invention concerns a sex-related proteinaceous material, encoded in nature by a sex-related gene, the invention also relates to the areas of sex linked congenital aberations, of embryonic selection techniques, in vitro fertilization techniques, vaccination and in ovo vaccination.

Bone marrow transplantation (BMT), one of the areas the invention is concerned with and the area from which the present invention originates, finds its application in the treatment of for instance severe aplastic anaemia, leukaemia and immune deficiency diseases.

In the early days of this technique many transplants failed through rejection of the graft by the host. Transplants that did succeed, however often led to an immune response by lymphocytes present in the graft against various tissues of the host (Graft versus Host Disease (GvHD)). It is now known that the GvHD response is mainly due to the presence of major H antigens which present a transplantation barrier. Therefor it is now routine practice to graft only HLA-matched materials (either from siblings or unrelated individuals) resulting in a much improved rate of success in bone marrow transplantation. However, despite this improvement, as well as improvements in pretransplantation chemotherapy or radiotherapy and the availability of potent immunosuppressive drugs, about 20-70% of the treated patients still suffer from GvHD (the percentage is age and bone marrow donor dependent). To avoid GvHD it has been suggested to remove the cells (mature T cells) causing said reaction from the graft. This however often leads to graft failure or to recurrence of the original disease. The

cells responsible for GvHD are also the cells which often react against the original aberrant cells in for instance leukaemia (Graft versus Leukaemia response).

Since BMT is nowadays only carried out with HLA matched grafts, the GvHD which still occurs must be caused by another group of antigens. It is very likely that the group of so called minor H antigens (mHag), which are non-MHC encoded histocompatibility antigens (unlike the major H antigens) are at least partially responsible for the remaining incidence of GvHD.

mHag's have originally been discovered in congeneic strains of mice in tumor rejection and skin rejection studies. In mice, the use of inbred strains has shown that mHag are encoded by almost 50 different allelically polymorphic loci scattered throughout the genome (24). In humans, mHag have been shown to exist, although their overall number and complexity remains uncertain. One of the better known, though unidentified minor histocompatibility antigens is the H-Y antigen. In the first report of H-Y as a transplantation antigen Eichwald and Silmser observed that within two inbred strains of mice, most of the male-to female skin grafts were rejected, whereas transplants made in other sex combinations nearly always succeeded (1). The term H-Y antigen was introduced by Billingham and Silvers (2) because the male specific antigen can function as a classical transplantation antigen responsible for homograft rejection.

Alloimmunity to human H-Y was first demonstrated in a female patient with aplastic anaemia who was given bone marrow from her HLA-identical brother. After a period of transient chimaerism the graft was rejected. At this time after grafting her lymphocytes showed unambiguously strong MHC restricted cytotoxic T cell (CTL) responses specific for male HLA-A2 positive target cells (3,4). The clinical case not only evidenced that H-Y can function as a transplantation barrier in man as well, but also that the recognition of the human male specific minor Histocompatibility antigen (mHag) was MHC restricted (4). The clinical relevance of H-Y as alloantigen

is demonstrated especially in bone marrow transplantation (BMT) where sex-mismatch is one of the risk factors associated with rejection (3,4,5) or Graft-versus-Host-Disease (6,7). Sensitization to the H-Y antigen extends to organ transplantation (8-11), bloodtransfusion (12) and pregnancy (13), wherein MHC restricted T cell responses to the mHag H-Y in association with different MHC molecules are observed. To understand the impact of mHag H-Y on the outcome of organ- and bone marrow grafting we earlier studied its tissue distribution. CTL mediated lysis of tissue-derived cell and cultured cell lines of several human tissues demonstrated an ubiquitous expression (11,14,16).

In search for the biological function of the gene encoding the mHag H-Y, our earlier studies analyzing lymphocytes from sex chromosomal abnormalities with our HLA restricted H-Y specific CTL clones revealed that absence of the mHag H-Y correlated with the XO and XX karyotype (17). Subsequent studies combining DNA, and functional expression with our CTL clones analyzing lymphocytes from individuals with Y chromosomal deletions, assigned the H-Y gene encoding the mHag H-Y to a portion of interval 6 (18), to a region covering the proximal segment of the Yq euchromatin, on the long arm of the Y chromosome (19).

Besides the role of H-Y as transplantation antigen, the human Y gene controlling the expression of the mHag H-Y is possibly also functioning as a gene controlling spermatogenesis. Agulnik et al. (20) recently identified a new murine Y chromosome gene, designated Smcy, controlling spermatogenesis as well the expression of the murine male specific mHag H-Y. The Smyc gene appears to be conserved on the Y chromosome in mouse, man and even in marsupials (20). It is notable that recent studies from our laboratories show recognition of the human HA-2 and H-Y peptides on non human primates cells, transfected with human class I genes, by our human HA-2 and H-Y specific class I restricted CTL clones (21).

Until recently, little was known about the molecular nature of the mHag gene products. Recent evidence was obtained revealing that the non-sexlinked human mHag HA-2 represents a short peptide originating from a member of the non-filament-forming class I myosin family (22). However, no information exists on the amino-acid sequence nor on the protein of the male specific mHag H-Y.

Aiming at the identification of the human H-Y peptide, we used the HLA-B7 restricted CTL clone "5W4" (12). Clone 5W4 originates from a female aplastic anemia patient who had received mutiple transfusions (12,23).

Besides the HLA-B7 H-Y specific CTL clone, we earlier characterized HLA-A2 as well as HLA-A1 H-Y specific CTL clones (23).

We used a CD8 positive HLA-A2.1 restricted H-Y specific CTL clone, designated "1R35" (23). Besides, we also previously characterized a CD4 positive HLA-A2.1 restricted H-Y specific cytotoxic as well as proliferative T cell clone, designated as "R416" (41).

We aimed at identification of the human H-Y peptide recognized by the HLA-A2.1 restricted H-Y specific T cell clones IR35 and R416. The same methodology as applied for the identification of the HLA-B7 restricted H-Y peptide was used.

The invention thus provides a (poly)peptide comprising a T-cell epitope obtainable from the minor Histocompatibility antigen H-Y comprising the sequence SPSVDKARAEL or FIDSYICQV or a derivative of either of these having similar immunological properties.

The two sequences specified are encoded by the SMCY gene. The first sequence is the one found using the HLA-B7 restricted H-Y specific T-cell clone, The second is the one found using the HLA-A2.1 restricted clones.

The way these sequences are obtained is described herein. An important part of this novel method of arriving at said sequences is the purification and the choice of the starting material. Said novel method is therefor also part of the scope of this invention. However, now that the sequence is known, it

is of course no longer necessary to follow that method, because the peptides can easily be made synthetically, as is well known in the art. Since routine techniques are available for producing synthetic peptides, it is also within the skill of the art to arrive at analogs or derivatives of the explicitly described peptides, which analogs and/or derivatives may have the same or at least similar properties and or activity. On the other hand analogs which counteract the activity of the explicitly described peptides are also within the skill of the art, given the teaching of the present invention. Therefor derivatives and/or analogs, be it of the same or different length, be it agonist or antagonist, be it peptide-like or peptidomimetic, are part of the scope of this invention.

A preferred embodiment of the present invention are the peptides with the sequences SPSVDKARAEL and/or FIDSYICQV. This does not imply that other peptides are not suitable. This will for a large part depend on the application and on other properties of the peptides, which were not all testable within the scope of the present invention.

The peptides and other molecules according to the invention find their utility in that they may be used to induce tolerance of the donor immune system in H-Y negative donors, so that residual peripheral blood lymphocytes in the eventually transplanted organ or the bone marrow, as it may be do not respond to host H-Y material in an H-Y positive recipient. In this way GvHD may be prevented. On the other hand tolerance may be induced in H-Y negative recipients in basically the same way, so that upon receipt of an organ or bone marrow from an H-Y positive donor no rejection on the basis of the H-Y material occurs.

For tolerance induction very small doses can be given repeatedly, for instance intravenously, but other routes of administration may very well be suitable too. Another possibility is the repeated oral administration of high doses of the peptides. The peptides may be given alone, or in combination with other peptides, or as part of larger

molecules, or coupled to carrier materials in any suitable excipients.

Further applications of the peptide or derivatives thereof lie in the prophylactic administration of such to transplanted individuals to prevent GvHD. This can be done with either agonists, possibly in combination with an adjuvant, or with antagonists which may block the responsible cells. This can be done with or without the concomittant administration of cytokines.

Furthermore the peptides or antibodies thereto can be used in so called "magic bullet" applications, whereby the peptide or the antibody is coupled to a toxic substance to eliminate certain subsets of cells.

Diagnostic applications are clearly within the skill of the art. They include, but are not limited to H-Y typing, detection of genetic aberrancies and the like.

Other therapeutical applications of the peptide include the induction of tolerance to H-Y proteins in H-Y related (auto)immune diseases, such as possibly in Rheumatoid arthritis. On the other hand they may be used in vaccines in H-Y related (auto)immune diseases.

For the sake of illustration a number of applications is cited below.

The H-Y peptide or its derivatives can be used to prevent harmful reaction of the recipient towards the donor or vice versa; in all forms of transplantation i.e. organs, tissues and bone marrow. Assuming that residual donor peripheral blood lymphocytes (PBL)'s in the transplanted organ could react with and/or against host PBL's and even could cause GvHD, the H-Y peptide could be used to induce tolerance in living organ (kidney, liver, gut, skin) of H-Y negative donors for H-Y positive patients. In bone marrow transplantation, the H-Y peptide (given alone or in combination with other peptides) can be used to induce tolerance in the living bone marrow donor. The peptide(s) can be given orally, intravenous or otherwise.

In all forms of organ (including cornea), tissue (including heartvalves and skin) and bone marrow transplantation with living or cadaveric donors, the H-Y peptide could be used to induce tolerance in H-Y negative recipients of organ and tissue transplants from H-Y positive donors. In case of bone marrow transplantation, tolerance must be induced in female donors for male recipients. The tolerance induction can be achieved by clinical application of the H-Y peptide systematically, i.v., locally, orally, as eye-drops.

The H-Y peptides could act in a non-allelic restricted manner (thus promiscuous) implicating that its applicability to inducing tolerance is not restricted to the HLA type of the female donors and female recipients and donors .

The H-Y peptides or their derivatives can be applied to generate reagents and/or medicine. They can be used as Graft-versus-Host disease and rejection prophylaxis administration to the transplanted individual either with or without adjuvant of

a) a H-Y peptide

b) H-Y peptide analogues, including left or right turning peptides

c) H-Y peptide antagonists

Usage of the H-Y sequence information to generate, for immunomodulatory purposes:

a) anti-idiotypic T cells

b) anti-idiotypic B cells

c) human monoclonal antibodies

The H-Y peptides or their derivatives can be used as a marker for sex linked congenital or other diseases.

They can be used for the generation of a genetic probe enabling screening for the congenital sex-linked disorders.

The genetic probe can be used for genetic counseling, population genetics and pre-natal diagnostic.

The defect can be repaired by genetic engineering.

The peptides and other molecules according to the invention can also be used for the production of anti-conceptive drugs.

Furthermore the peptides and other molecules according to the invention can be used for the production of cytotoxic T lymphoctes (CTL) with specificity for the H-Y sequence.

The H-Y specific CTL can be used for selection of male embryos in X linked recessive disorders.

The invented molecules can be applied to generate reagents and/or medicine for

a) determination of foetal erythrocytes in maternal circulation.
b) intra uterine diagnostics
c) use prior to implantation for *in vitro* fertilization.
d) determination of chimerism.

Veterinary applications include:

a) embryonic selection.
b) *in vitro* fertilization.
c) vaccination and in ovo vaccination
d) anti-conception.

On the basis of the peptides described herein genetic probes can be produced which can be used to screen for the gene encoding the protein. On the other hand such probes may be useful in detection kits as well. On the basis of the peptides described herein anti-idiotypic B cells and/ or T cells and antibodies can be produced. All these embodiments have been made possible by the present disclosure and therefor are part of the present invention.

The techniques to produce these embodiments are all within the skill of the art.

Dose ranges of peptides and antibodies and/or other molecules according to the invention to be used in the therapeutical applications as described herein before are usually designed on the basis of rising dose studies in the clinic. The doses for peptides may lie between about 0.1 and 1000 µg per kg bodyweight, preferably between 1 and 10 µg per kg bodyweight.

Detailed description of the invention.

As with other mHag, the recognition of H-Y by T lymphocytes is MHC-restricted (3,24,25), and it has been shown that some H-Y antigens are peptides derived from cellular proteins that are presented on the cell surface in association with MHC class I molecules (26). We have developed a technique for the identification of individual peptides that are bound to MHC molecules and recognized as antigens by T cells. By combining microcapillary liquid chromatography/electrospray ionization mass spectrometry with T cell epitope reconstitution assays, we previously identified peptide antigens recognized by T cells specific for human melanoma (27), human xenografts (28), and a non-sex-linked human mHag (22). We now report the identification of a peptide antigen recognized by a human cytotoxic T lymphocyte (CTL) clone that is H-Y specific and restricted by the class I MHC molecule HLA-B7, as well as a peptide antigen that is recognized by two HLA-A2.1 restricted CTL clones.

To isolate endogenously processed H-Y peptides, HLA-B7 molecules were purified by affinity chromatography from the H-Y positive, B lymphoblastoid cell line, JY (29). The associated peptides were extracted in acid and separated from high molecular weight material by ultrafiltration as previously described (31), and subsequently fractionated by reverse-phase high-performance liquid chromatography (HPLC) (27). Aliquots of each fraction were incubated with HLA-B7 positive, H-Y negative T2-B7 target cells in order to assay for the ability to reconstitute the epitope recognized by an HLA-B7-restricted, H-Y specific CTL clone, 5W4 (ref. 12). A single peak of reconstituting activity was observed (Fig. 1A, fraction 28 and 29), which was rechromatographed using a different organic modifier. Although a single active peak of reconstituting activity was also observed from this separation (Fig 1B, fraction 14, 15 and 16), it still contained more than 100 distinct peptide species, as assessed by electrospray ionization tandem mass spectrometry.

To identify active H-Y peptides in this mixture, we applied each active fraction separately to a microcapillary HPLC column and split the effluent following the separation (11): Four-fifths of the effluent was directed into the mass spectrometer for analysis, while one-fifth was simultaneously directed into a 96-well microtiter plate for a subsequent epitope reconstitution assay. The amount of the H-Y sensitizing activity in each well was correlated to signals observed in the mass spectrum, and therefore to the abundance of different peptide species. By comparing the profile of H-Y activity and the ion abundance data (Fig. 2), we were able to identify an (M+3H)+3 ion at a mass-to-charge ratio (m/z) of 391 (neutral molecular mass =1171), whose abundance correlated with the amount of H-Y epitope reconstituting activity. Further confirmation of the importance of peptide 1171 was provided by the demonstration that a peptide with an identical mass and collision-activated dissociation (CAD) spectrum was also present in HLA-B7 associated peptides extracted from a second H-Y positive B lymphoblastoid line, DM, but absent from a spontaneous H-Y antigen loss variant of this cell, DM(-) (33).

Assignment of a complete amino acid sequence to the 1171 peptide from the CAD mass spectrum recorded at the 20 fmol level proved difficult due to the absence of high mass fragment ions containing the amine terminus (b-type ions). A series of single and / or doubly charged fragment ions containing the carboxyl terminus (y-type ions) identified the C-terminal residue as either L or I and the first six amino acids as SPSVDK. The difference in molecular mass between this partial sequence and that of the full length peptide suggested the presence of four additional residues, for a total length of 11. Since the candidate peptide existed exclusively in the gas phase as an (M+3H)+3 ion, and underwent mass shifts of 42 and 84 Da on conversion to the corresponding methyl ester and acetylated derivative, respectively, two of the remaining residues were assigned as R and either D or E. Only two combinations of four residues (AREA and GRDV) meet the above

criteria and satisfy the missing mass of 427 Da. CAD spectra recorded on synthetic peptides suggested that R could not be located at either position 7 or 10. Data bases were searched for proteins containing peptides with these characteristics, and a sequence consistent at 9 out of 11 positions was found in residues 909-919 of the protein encoded by a gene called XE169 or SMCX (34), which is located on the X chromosome. A homolog of SMCX, called SMCY, is located on the Y chromosome (20). This protein (35) contains a sequence (residues 902-912) that is consistent at 11 out of 11 positions, and has the expected mass of 1171 Da. A CAD mass spectrum recorded on the naturally processed material after conversion of the R residue to ornithine confirmed that its sequence was identical to that found in the SMCY protein (Fig. 3).

In the same manner as described above for the HLA-B7 restricted T-cell clone, the peptide recognized by two HLA-A2.1 T-cell clones was identified. In short the HLA-A2.1 restricted H-Y specific T cell clone R416 recognizes HPLC fraction 34, the HLA-A2.1 restricted H-Y specific T clone 1R35 recognizes HPLC fractions 36 and 39 (figure 6). The amino acid sequence analyses and H-Y reconstitution assays demonstrate that both HLA-A2.1 restricted H-Y specific T cell clones recognize peptide sequence FIDSYICQV with a m/z ratio of 544 or the cystinylated form of the same peptide with a m/z ratio of 604.

A synthetic peptide corresponding to the 11 residue SMCY sequence (SPSVDKARAEL) was found to sensitize T2-B7 cells for recognition by the H-Y specific CTL clone. Half-maximal lysis was achieved at a peptide concentration of 10 pM (Fig. 4). The corresponding peptide derived from the sequence of the X chromosomal homolog, SMCX, has substitutions of A for S at position 3 and Q for R at position 8. Although this peptide also was able to sensitize T2-B7 cells for recognition, comparable levels of killing were only achieved by using a 10,000-fold higher peptide concentration. Binding studies showed that the concentration of the SMCY peptide that inhibited the binding of an iodinated standard peptide to

purified HLA-B7 by 50% (IC50) was 34 nM, while the IC50 for the SMCX peptide was 140 nM (Fig. 5). Thus, the significant difference in the ability of the SMCY and SMCX peptides to sensitize targets for T cell recognition is almost entirely due to the fine specificity of the T cell receptor, rather than to differences in MHC binding affinities. The SMCX peptide is also present in naturally processed peptide extracts of HLA-B7, although its abundance is only 25% of that of the SMCY peptide (33). Based on all of this information, we conclude that the peptide epitopes representing the HLA-B7 restricted H-Y antigen is derived from the protein encoded by SMCY, which is also true for the HLA-A2.1 recognized peptide, also encoded by SMCY.

The location of the SMCY gene and the control of its expression fit well with those expected of the H-Y antigen based on previous work. Deletion mapping in humans has placed the HY locus to a portion of interval 6 on the long arm of the human Y chromosome (18), and SMCY maps to this same interval (20). H-Y antigens are expressed ubiquitously in different tissues (5,15), and expression of SMCY has been detected in all male tissues tested (20). One interesting issue is whether the H-Y epitope peptides presented by other MHC molecules will also be derived from SMCY. SMCY and SMCX are 85% identical at the amino acid sequence level, and the SMCX gene is expressed ubiquitously from both the active and the inactive X chromosomes in both mice and human (34,36). Therefore, self-tolerance to SMCX will limit the number of SMCY peptides that could give rise to H-Y epitopes in association with different MHC molecules. On the other hand, SMCY contains almost 1500 residues, and the over 200 amino acid sequence differences between it and SMCX are scattered relatively uniformly throughout its length. Thus, there is the potential to generate a large number of distinct SMCY-specific peptides as H-Y epitopes. It is still an open question whether the H-Y epitope peptides presented by other MHC molecules are also derived from SMCY. Genetic mapping of the mouse Y chromosome has suggested at least two and up to five distinct loci

encoding H-Y antigens (37). Interestingly, a murine H-Y epitope restricted by H-2Kk has also been shown to be derived from the murine Smcy protein (38). The demonstration that two H-Y epitopes from either mouse or human are derived from the same protein makes SMCY the prime target in searching other H-Y epitopes.

The identification of the protein that gives rise to an H-Y antigen culminates 40 years of uncertainty regarding its origin. However, the function of SMCY, as well as the homologous SMCX, remains unclear. Both proteins share significant sequence homology to retinoblastoma binding protein 2, which has been suggested to be a transcription factor (39). Nonetheless, this and other H-Y specific peptides are candidates for immunomodulatory approaches in bone marrow transplantation. They may also form the basis for genetic probes to be used for prenatal diagnosis in sex-linked congenital abnormalities, as well as for investigating minimal residual disease and chimerism.

Fig. 1.

Reconstitution of the H-Y epitope with HPLC fractionated peptides extracted from HLA-B7 molecules. (A) HLA-B7 molecules were immunoaffinity purified from 2x1010 H-Y positive JY cells. Peptides were eluted from B7 molecules with 10% acetic acid, pH 2.2, filtered through a 10 kD cut-off filter and fractionated on a C18 reverse phase column. Buffer A was 0.1% heptafluorobutyric acid (HFBA); buffer B was 0.1% HFBA in acetonitrile. The gradient consisted of 100% buffer A (0-20 min), 0 to 12% buffer B (20 to 25 min), and 12 to 50% buffer B (25 to 80 min) at a flow rate of 200 µl/min. 60 fractions of 200 µl each were collected from 20 to 80 min. (B) Fractions 28 and 29 from the separation shown in (A) were rechromatographed with the same acetonitrile gradient, but using trifluoroacetic acid (TFA) instead of HFBA as the organic modifier. For both panels, 3% of each peptide fractions were preincubated with 1,000 ^{51}Cr-labeled T2-B7 cells at room temperature for 2 hours. CTLs were then added at an effector to target ratio of 10 to 1, and further incubated at 37°C for 4 hours. Background lysis of T2-B7 by the CTL in the absence of any peptides was -3% in (A) and -4% in (B); positive control lysis of JY was 75% in (A) and 74% in (B).

Fig. 2.

Determination of candidate H-Y peptide by mass spectrometry combined with ^{51}Cr release assay. HPLC fraction 14 from the separation shown in Fig. 1B was chromatographed with an on-line microcapillary column effluent splitter as previously described (11,13) One-fifth of the effluent was deposited into µl of culture media in microtiter plate wells for analysis with CTLs as in Fig. 1. The remaining four-fifths of the material were directed into the electrospray ionization source, and mass spectra of the peptides deposited in each well were recorded on a triple-quadrupole mass spectrometer (Finnigan-MAT, San Jose, California).

(◊), H-Y epitope reconstitution activity measured as percent specific lysis; (■), abundance of peptide 1171 measured as ion current at m/z 391.

Fig. 3.

CAD mass spectrum of peptide 1171 after conversion the R residue to ornithine. Material from second dimension HPLC fraction 14 shown in Fig. 1B was treated with 70% hydrazine hydrate for 1 hour. The CAD mass spectrum was recorded on the (M+2H)+2 ion at m/z 566.

Fig. 4.

H-Y epitope reconstitution with synthetic peptides. Synthetic peptides were purified to homogeneity by reverse phase-HPLC on a Vydac C4 column. Purity was established on an analytical RP column and the quantity of each peptide was confirmed by comparing the area of the peak with that of a standard peptide. The identity of the peptides was confirmed by mass spectrometry. ^{51}Cr release was assayed at an effector to target ratio of 10 to 1 on T2-B7 cells that had been incubated with the indicated concentration of SMCY peptide SPSVDKARAEL (◊), or SMCX peptide SPAVDKAQAEL (■).

Fig. 5.

Binding of synthetic peptides to purified HLA-B7. HPLC-purified test peptides were assayed for the ability to inhibit the binding of the iodinated endogenous B7 peptide APRTYVLLL to purified HLA-B7 as previously described (40). (◊), SMCY peptide SPSVDKARAEL; (■)SMCX peptide SPAVDKAQAEL; (▲), APRTLVLLL, an endogenous peptide bound to HLA-B7; (**X**) LLDVPTAAV, an endogenous peptide bound to HLA-A2.1 as the negative control.

Fig. 6.

HLA-A2 molecules were immunoaffinity purified from 10^{10} DM cells. Peptides were eluted according to the methodology as described in legend to Fig. 1.

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PCT 0490

CLAIMS

1. A peptide constituting a T-cell epitope obtainable from the minor Histocompatibility antigen H-Y comprising the sequence SPSVDKARAEL or the sequence FIDSYICQV or a derivative of either of these peptides having similar immunological properties.

2. An immunogenic polypeptide obtainable from the minor Histocompatibility antigen H-Y comprising the sequence SPSVDKARAEL or the sequence FIDSYICQV or a derivative thereof.

3. Vaccine comprising an epitope or a polypeptide according to claim 1 or 2.

4. A pharmaceutical formulation comprising an epitope or a polypeptide according to claim 1 or 2.

5. Peptide or polypeptide according to claim 1 or 2 for use as a medicine.

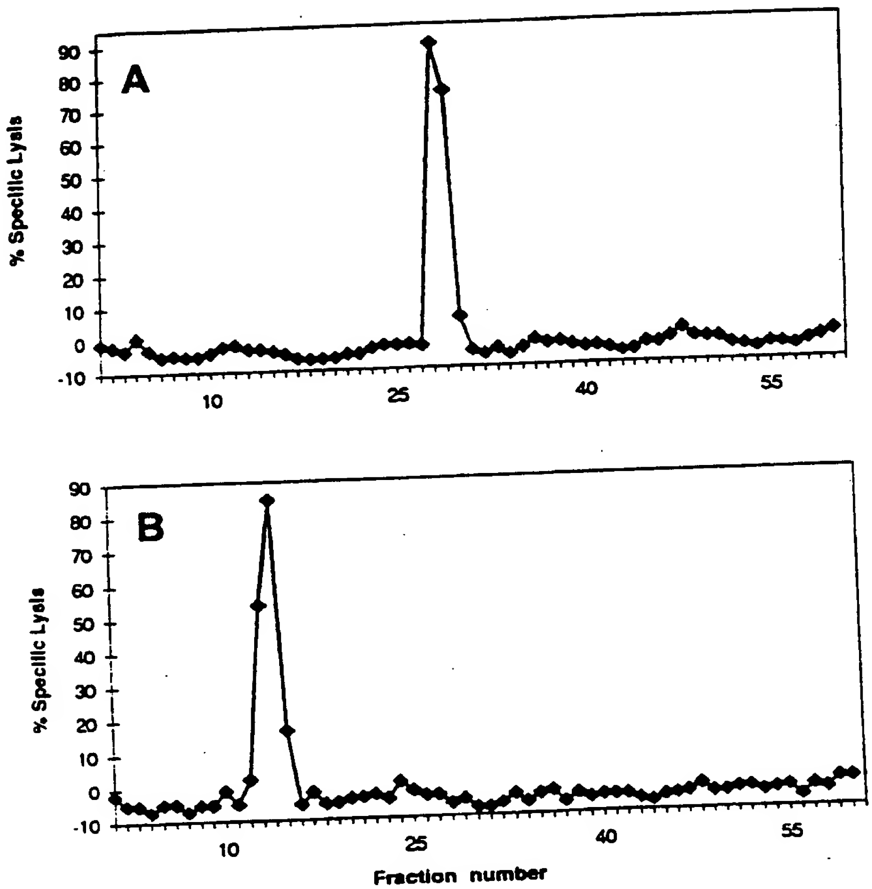
6. Use of a peptide or polypeptide according to claim 1 or 2 in the preparation of a medicament for the induction of tolerance for transplants to prevent rejection and/or Graft versus Host disease.

7. A method for the elimination of a subset of T-cells recognizing a peptide according to claim 1, wherein said peptide is provided with a toxic moiety.

8. Analog of the peptide according to claim 1, which is an antagonist for the activity of T cells recognizing said peptide.

9. Method for the generation of antibodies, T cell receptors, anti-idiotypic B-cells or T-cells, comprising the step of immunization of a mammal with a peptide or a polypeptide according to claim 1 or 2.

10. Antibodies, T-cell receptors, B-cells or T-cells obtainable by the method of claim 9.


A
B
% Specific Lysis
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80
70
60
50
40
30
20
10
0
-10
10
25
40
55
Fraction number

FIG. 1

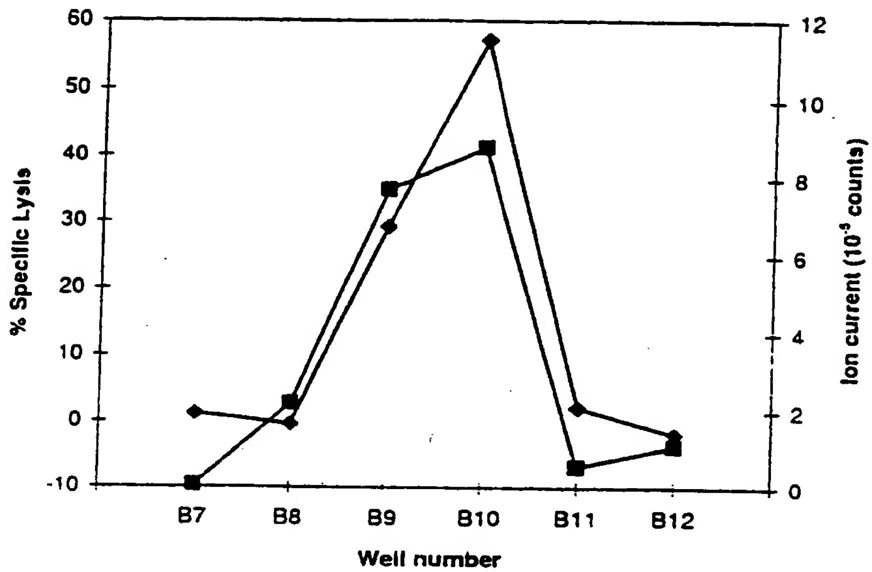

60
50
40
30
20
10
0
-10
% Specific Lysis
12
10
8
6
4
2
0
Ion current (10^{-5} counts)
B7
B8
B9
B10
B11
B12
Well number

FIG. 2

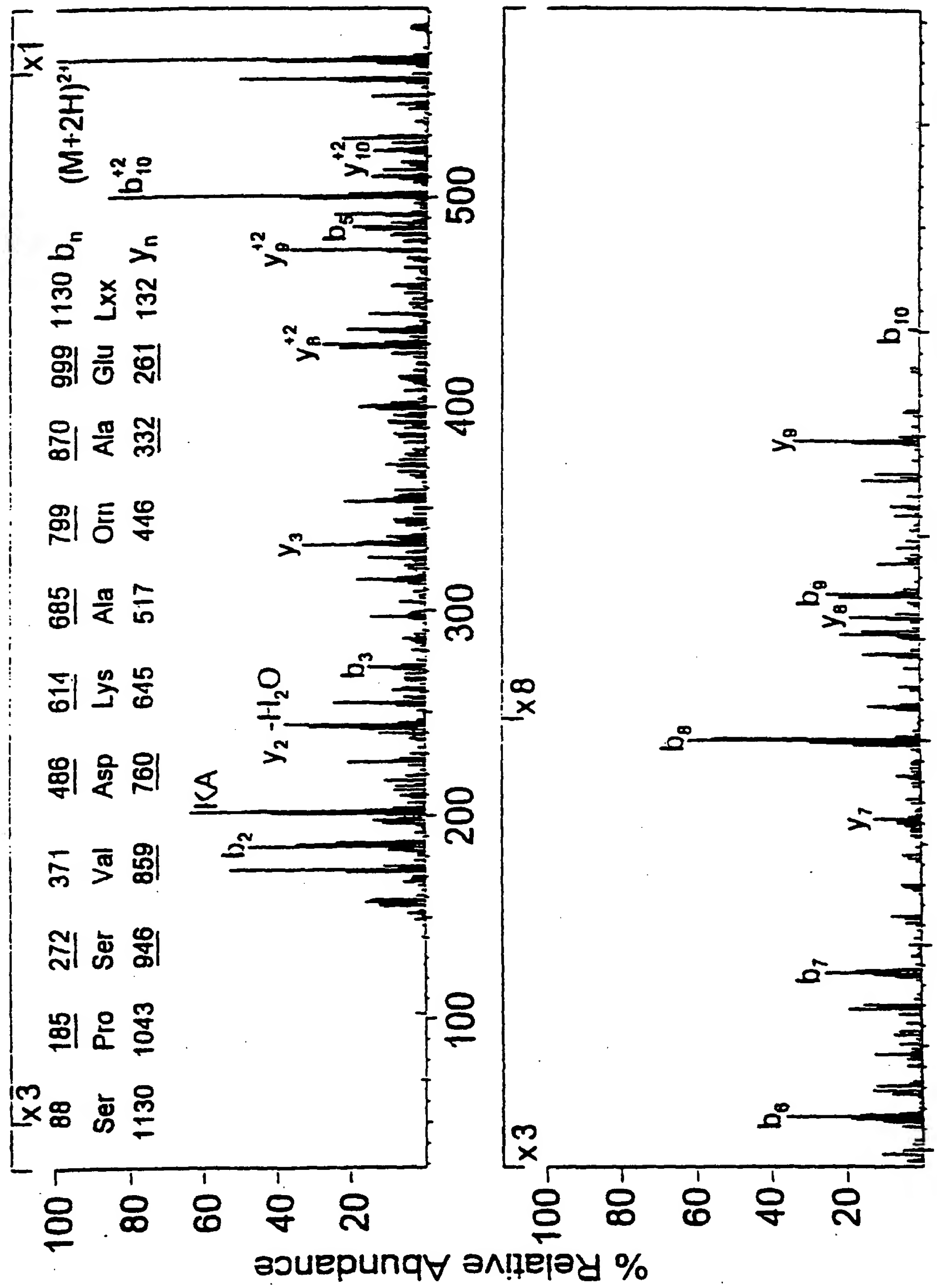

88 185 272 371 486 614 685 799 870 999 1130 bn
Ser Pro Ser Val Asp Lys Ala Orn Ala Glu Lxx
1130 1043 946 859 760 645 517 446 332 261 132 yn
(M+2H)2+
KA
x3
x1
x8
m/z
% Relative Abundance

Fig. 3

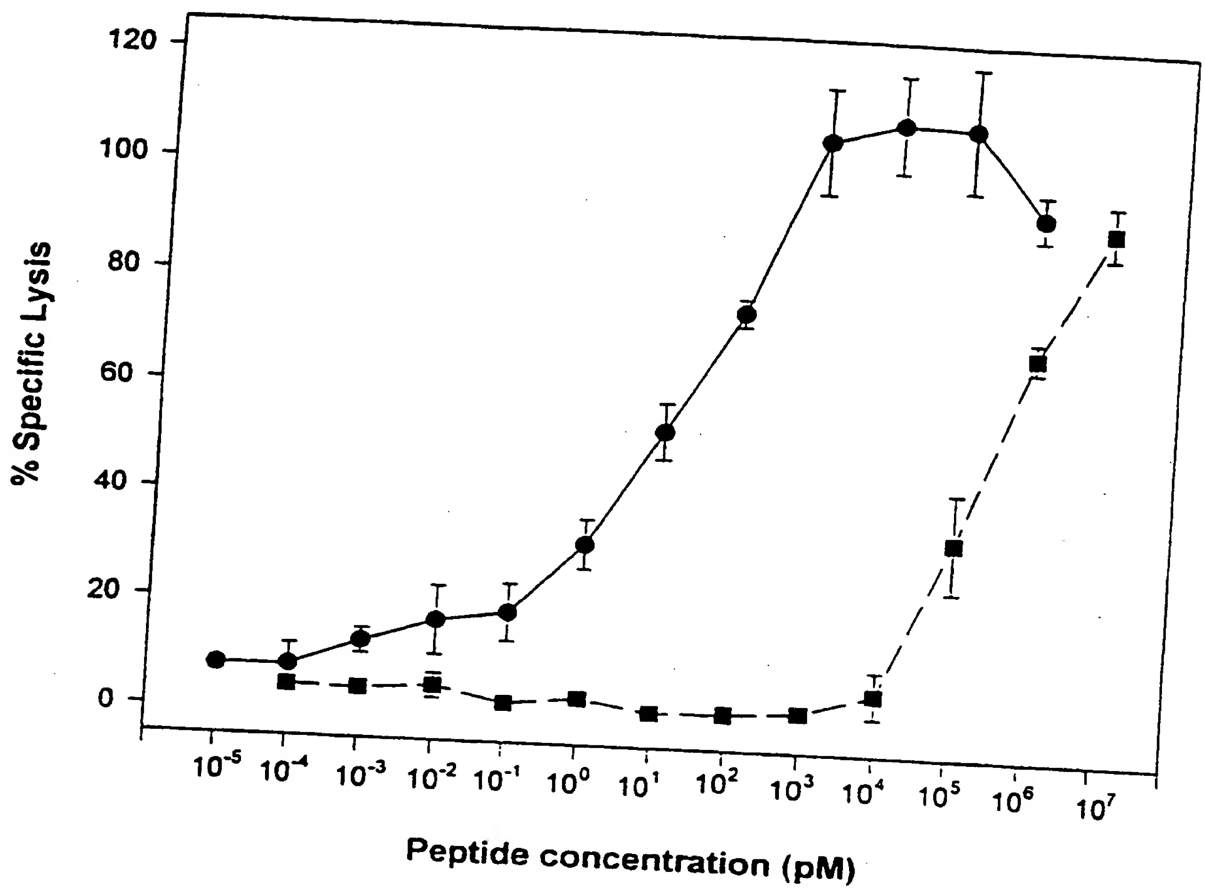

120
100
80
60
40
20
0
% Specific Lysis
10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3} 10^{4} 10^{5} 10^{6} 10^{7}
Peptide concentration (pM)

FIG. 4

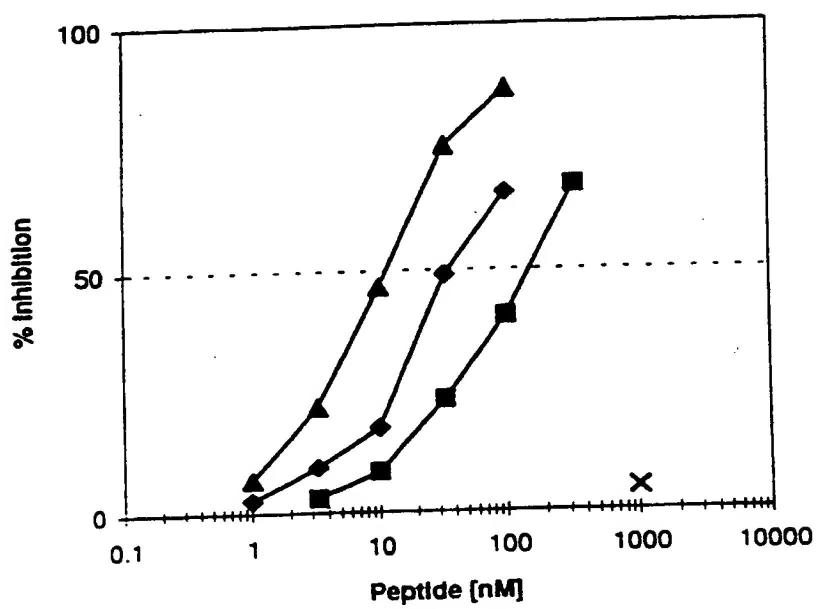
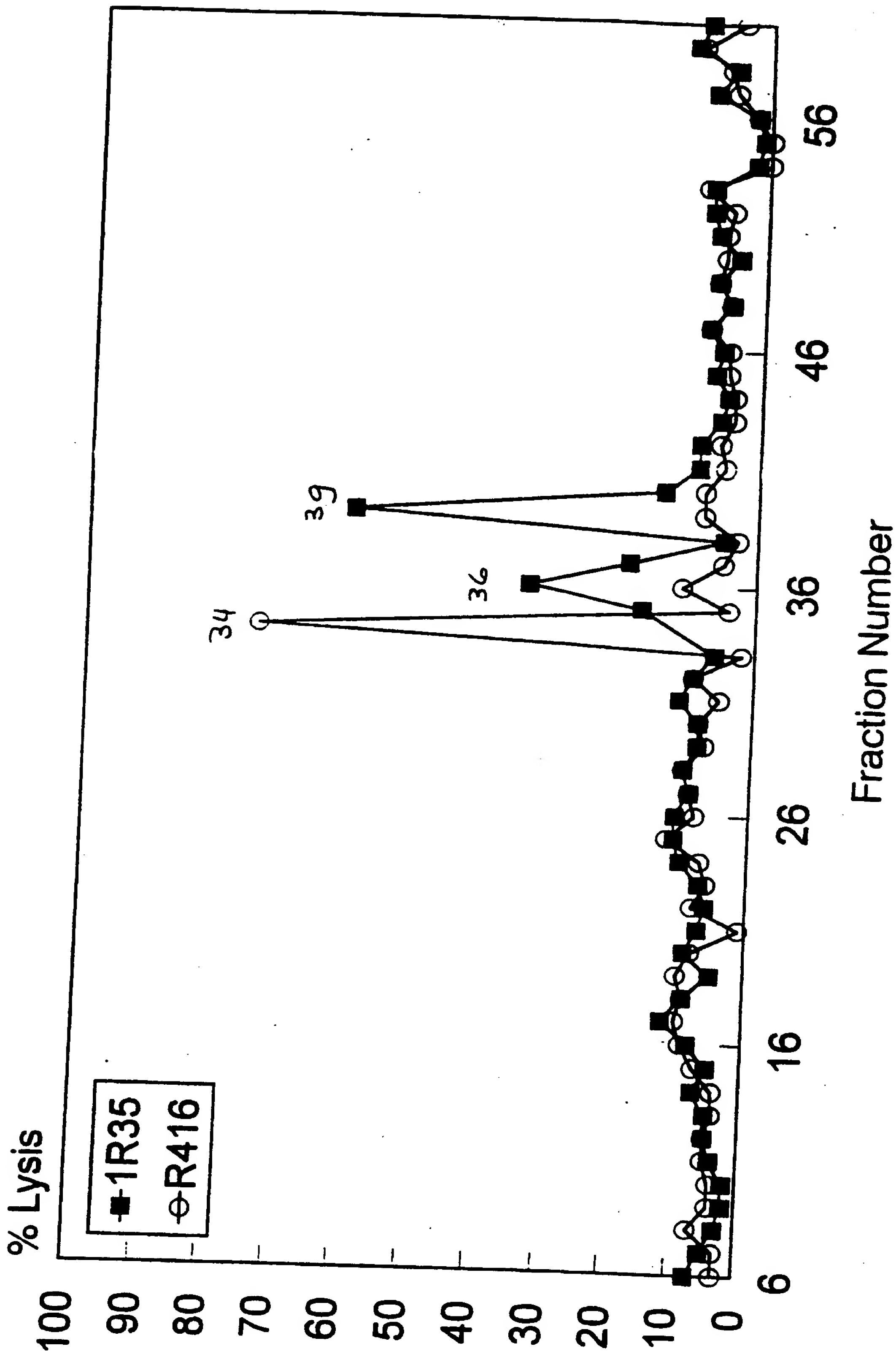


FIG. 5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R416
Fraction Number
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10
20
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40
50
60
70
80
90
100
6
16
26
36
46
56
34
36
39

FIG. 6

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 96/00307

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/705 C07K16/28 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HUM. MOL. GENET. (1994), 3(6), 873-78 CODEN: HMGEE5;ISSN: 0964-6906, 1994, XP000615278 AGULNIK, ALEXANDER I. ET AL: "A mouse Y chromosome gene encoded by a region essential for spermatogenesis and expression of male-specific minor histocompatibility antigens" cited in the application see page 876, left-hand column, paragraph 2 - right-hand column, paragraph 2; figure 3 ---	1,2

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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
16 December 1996	07.01.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Fuhr, C

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Intern nal Application No
PCT/NL 96/00307

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCIENCE, vol. 268, no. 5216, 9 June 1995, LANCASTER, PA US, pages 1476-1480, XP002021218 J.M.M. DEN HAAN ET AL.: "Identification of a Graft Versus Host Disease-Associated Human Minor Histocompatibility Antigen" cited in the application see the whole document ---	1-10
P,X	SCIENCE (WASHINGTON, D. C.) (1995), 269(5230), 1588-90 CODEN: SCIEAS;ISSN: 0036-8075, 15 September 1995, XP002021219 WANG, WEI ET AL: "Human H-Y: a male-specific histocompatibility antigen derived from the SMCY protein" see figures 2,3 ---	1,2
P,A	NATURE (LONDON) (1995), 376(6542), 695-8 CODEN: NATUAS;ISSN: 0028-0836, 24 August 1995, XP002021220 SCOTT, D. M. ET AL: "Identification of a mouse male-specific transplantation antigen, H-Y" see figure 3 -----	1,2

2

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:
   Please see Further Information sheet enclosed.

2. [ ] Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Applicati n No. PCT/NL 96/ 00307

FURTHER INFORMATION CONTINUED FR M PCT/ISA/210

Remark : As far as claim 7 is directed to a method of treatment of or diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.